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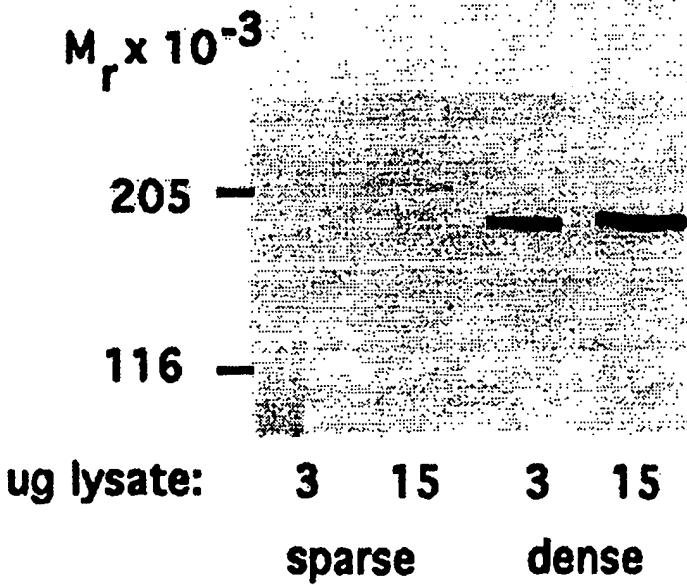
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(54) Title: DENSITY ENHANCED PROTEIN TYROSINE PHOSPHATASES

(57) Abstract

Novel Type III density enhanced protein tyrosine phosphatases are disclosed and exemplified by human DEP-1 enzyme. Polynucleotides encoding huDEP-1 are disclosed, along with methods and materials for production of the same by recombinant procedures. Binding molecules specific for DEP-1 are also disclosed as useful for modulating the biological activities of DEP-1.



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DENSITY ENHANCED PROTEIN TYROSINE PHOSPHATASES

FIELD OF THE INVENTION

The present invention relates generally to purified and isolated protein tyrosine phosphatase enzymes (PTPs) and polynucleotides encoding the same. PTPs of the invention are characterized by upregulated mRNA transcription and/or translation, or post-translational modification leading to increased total cellular enzyme activity as a function of increased cellular contact with neighboring cells. Such density enhanced PTPs are referred to as DEPTPs. An illustrative human Type III receptor-like density-enhanced protein tyrosine phosphatase has been designated huDEP-1.

BACKGROUND OF THE INVENTION

Protein tyrosine phosphorylation is an essential element in signal transduction pathways which control fundamental cellular processes including growth and differentiation, cell cycle progression, and cytoskeletal function. Briefly, the binding of growth factors, or other ligands, to a cognate receptor protein tyrosine kinase (PTK) triggers autophosphorylation of tyrosine residues in the receptor itself and phosphorylation of tyrosine residues in the enzyme's target substrates. Within the cell, tyrosine phosphorylation is a reversible process; the phosphorylation state of a particular tyrosine residue in a target substrate is governed by the coordinated action of both PTKs, catalyzing phosphorylation, and protein tyrosine phosphatases (PTPs), catalyzing dephosphorylation.

The PTPs are a large and diverse family of enzymes found ubiquitously in eukaryotes [Charbonneau and Tonks, *Ann. Rev. Cell Biol.* 8:463-493 (1993)]. Structural diversity within the PTP family arises primarily from variation in non-catalytic (potentially regulatory) sequences which are linked to one or more highly conserved catalytic domains. In general, soluble cytoplasmic PTP forms are termed non-receptor PTPs and those with at least one non-catalytic region that traverses the cell membrane are termed receptor-like PTPs (RPTPs).

- 2 -

A variety of non-receptor PTPs have been identified which characteristically possess a single catalytic domain flanked by non-catalytic sequences. Such non-catalytic sequences have been shown to include, among others, sequences homologous to cytoskeletal-associated proteins [Yang and Tonks, *Proc.Natl.Acad.Sci.(USA)* 88:5949-5953 (1991)] or to lipid binding proteins [Gu, *et al.*, *Proc.Natl.Acad.Sci.(USA)* 89:2980-2984 (1992)], and/or sequences that mediate association of the enzyme with specific intracellular membranes [Frangioni *et al.*, *Cell* 68:545-560 (1992)], suggesting that subcellular localization may play a significant role in regulation of PTP activity.

Analysis of non-catalytic domain sequences of RPTPs suggests their involvement in signal transduction mechanisms. However, binding of specific ligands to the extracellular segment of RPTPs has been characterized in only a few instances. For example, homophilic binding has been demonstrated between molecules of PTP μ [Brady-Kalnay, *et al.*, *J.Cell.Biol.* 122:961-972 (1993)] *i.e.*, the ligand for PTP μ expressed on a cell surface is another PTP μ molecule on the surface of an adjacent cell. Little is otherwise known about ligands which specifically bind to, and modulate the activity of, the majority of RPTPs.

Many receptor-like PTPs comprise an intracellular carboxyl segment with two catalytic domains, a single transmembrane domain and an extracellular amino terminal segment [Krueger *et al.*, *EMBO J.* 9:3241-3252 (1990)]. Subclasses of RPTPs are distinguished from one another on the basis of categories or "types" of extracellular domains [Fischer, *et al.*, *Science* 253:401-406 (1991)]. Type I RPTPs have a large extracellular domain with multiple glycosylation sites and a conserved cysteine-rich region. CD45 is a typical Type I RPTP. The Type II RPTPs contain at least one amino terminal immunoglobulin (Ig)-like domain adjacent to multiple tandem fibronectin type III (FNIII)-like repeats. Similar repeated FNIII domains, believed to participate in protein:protein interactions, have been identified in receptors for IL2, IL4, IL6, GM-CSF, prolactin, erythropoietin and growth hormone [Patty, *Cell* 61:13-14 (1992)].

- 3 -

The leukocyte common antigen-related PTP known as LAR exemplifies the Type II RPTP structure [Streuli *et al.*, *J.Exp.Med.* 168:1523-1530 (1988)], and, like other Type II RPTPs, contains an extracellular region reminiscent of the NCAM class of cellular adhesion molecules [Edelman and Crossin, *Ann.Rev.Biochem.* 60:155-190 (1991)]. The Type III RPTPs, such as HPTP β [Krueger *et al.*, *EMBO J.* 9:3241-3252 (1990)], contain only multiple tandem FNIII repeats in the extracellular domain. The Type IV RPTPs, for example RPTP α [Krueger *et al.* (1990) *supra*], have relatively short extracellular sequences lacking cysteine residues but containing multiple glycosylation sites. A fifth type of RPTP, exemplified by PTP γ [Barnes, *et al.*, *Mol.Cell.Biol.* 13:1497-1506 (1993)] and PTP ζ [Krueger and Saito, *Proc.Natl.Acad.Sci.(USA)* 89:7417-7421 (1992)], is characterized by an extracellular domain containing a 280 amino acid segment which is homologous to carbonic anhydrase (CAH) but lacks essential histidine residues required for reversible hydration of carbon dioxide.

FNIII sequences characteristically found in the extracellular domains of Type II and Type III RPTPs comprise approximately ninety amino acid residues with a folding pattern similar to that observed for Ig-like domains [Bork and Doolittle, *Proc.Natl.Acad.Sci.(USA)* 89:8990-8994 (1992)]. Highly conserved FNIII sequences have been identified in more than fifty different eukaryotic and prokaryotic proteins [Bork and Doolittle, *Proc.Natl.Acad.Sci.(USA)* 89:8990-8994 (1992)], but no generalized function has been established for these domains. Fibronectin itself contains fifteen to seventeen FNIII domain sequences, and it has been demonstrated that the second FNIII domain (FNIII₂) contains a binding site for heparin sulphate proteoglycan [Schwarzauer, *Curr.Opin.Cell Biol.* 3:786-791 (1991)] and that FNIII₁₃ and FNIII₁₄ are responsible for heparin binding through ionic interactions [Schwarzauer, *Curr.Opin.Cell Biol.* 3:786-791 (1991)]. Perhaps the best characterized interaction for a fibronectin FNIII domain involves FNIII₁₀ which is the major site for cell adhesion [Edelman and Crossin, *Ann.Rev.Biochem*

- 4 -

60:155-190 (1991); Leahy, *et al.*, *Science* 258:987-991 (1992); Main, *et al.*, *Cell* 71:671-678 (1992)]. FNIII₁₀ contains the amino acid sequence Arg-Gly-Asp (RGD) which is involved in promoting cellular adhesion through binding to particular members of the integrin superfamily of proteins.

5 Characteristics shared by both the soluble PTPs and the RPTPs include an absolute specificity for phosphotyrosine residues, a high affinity for substrate proteins, and a specific activity which is one to three orders of magnitude in excess of that of the PTKs *in vitro* [Fischer, *et al.*, *Science* 253:401-406 (1991); Tonks, *Curr. Opin. Cell. Biol.* 2:1114-1124 (1990)]. This latter 10 characteristic suggests that PTP activity may exert an antagonistic influence on the action of PTKs *in vivo*, the balance between these two thus determining the level of intracellular tyrosine phosphorylation. Supporting a dominant physiological 15 role for PTP activity is the observation that treatment of NRK-1 cells with vanadate, a potent inhibitor of PTP activity, resulted in enhanced levels of phosphotyrosine and generation of a transformed cellular morphology [Klarlund, *Cell* 41:707-717 (1985)]. This observation implies potential therapeutic value for PTPs and agents which modulate PTP activity as indirect modifiers of PTK activity, and thus, levels of cellular phosphotyrosine.

Recent studies have highlighted aspects of the physiological 20 importance of PTP activity. For example, mutations in the gene encoding a non-receptor hematopoietic cell protein tyrosine phosphatase, HCP, have been shown to result in severe immune dysfunction characteristic of the *motheaten* phenotype in mice [Schultz, *et al.*, *Cell* 73:1445-1454 (1993)]. Under normal conditions 25 HCP may act as a suppressor of PTK-induced signaling pathways, for example, the CSF-1 receptor [Schultz, *et al.*, *Cell* 73:1445-1454 (1993)]. Some PTP enzymes may be the products of tumor suppressor genes and their mutation or deletion may contribute to the elevation in cellular phosphotyrosine associated with certain neoplasias [Brown-Shimer, *et al.*, *Cancer Res.* 52:478-482 (1992); Wary, *et al.*, *Cancer Res.* 53:1498-1502 (1993)]. Mutations observed in the gene

- 5 -

for RPTP γ in murine L cells would be consistent with this hypothesis [Wary, *et al.*, *Cancer Res.* 53:1498-1502 (1993)]. The observation that the receptor-like PTP CD45 is required for normal T cell receptor-induced signalling [Pingel and Thomas, *Cell* 58:1055-1065 (1989)] provides evidence implicating PTP activity as a positive mediator of cellular signalling responses.

Normal cells in culture exhibit contact inhibition of growth, *i.e.*, as adjacent cells in a confluent monolayer touch each other, their growth is inhibited [Stoker and Rubin, *Nature* 215:171-172 (1967)]. Since PTKs promote cell growth, PTP action may underlie mechanisms of growth inhibition. In Swiss mouse 3T3 cells, a phosphatase activity associated with membrane fractions is enhanced eight-fold in confluent cells harvested at high density as compared to cells harvested from low or medium density cultures [Pallen and Tong, *Proc. Natl. Acad. Sci. (USA)* 88:6996-7000 (1991)]. This elevated activity was not observed in subconfluent cell cultures brought to quiescence by serum deprivation. The enhanced phosphatase activity was attributed to a 37 kD protein, as determined by gel filtration, but was not otherwise characterized. Similarly, PTPs have been directly linked to density arrest of cell growth; treatment of NRK-1 cells with vanadate was able to overcome density dependent growth inhibition and stimulate anchorage independent proliferation, a characteristic unique to transformed, or immortalized, cells [Klarland, *Cell* 41:707-717 (1985); Rijken, *et al.*, *J. Cell Physiol.* 154:343-401 (1993)].

In contrast to these observations, PCT Publication No. WO 94/03610 discloses a transmembrane PTP, termed PTP35, the steady state mRNA level of which was observed to be at a maximum in actively growing cells. Little or no PTP35 mRNA expression was detected in confluent cell. This mode of regulation was also observed in mouse 3T3 cells. Thus, two RPTPs in the same cell type apparently participate in opposing processes, with one (PTP35) contributing to cellular growth and the other (the 35 kD PTP of Pallen and Tong) contributing to cellular quiescence.

- 6 -

Interestingly, transcription of Type II RPTP LAR messenger RNA has been demonstrated to be upregulated in confluent fibroblast cell culture [Longo, *et al.*, *J. Biol. Chem.* 268:26503-26511 (1993)]. LAR is proteolytically processed to generate a mature protein that is a complex of two non-covalently associated subunits, one containing the majority of the cell adhesion molecule-like extracellular domain [Yu, *et al.*, *Oncogene* 7:1051-1057 (1992); Streuli, *et al.*, *EMBO J.* 11:897-907 (1992)] and which is shed as cells approach confluence [Streuli, *et al.*, *EMBO J.* 11:897-907 (1992)]. These observations lead to speculation regarding PTP involvement in modulation of cytoskeletal integrity, as well as other related cellular phenomena such as transformation, tumor invasion, metastasis, cell adhesion, and leukocyte movement along and passage through the endothelial cell layer in inflammation. The therapeutic implications are enormous for modulators of PTP activity which are capable of regulating any or all of these cellular events.

There thus exists a need in the art to identify members of the PTP family of enzymes and to characterize these proteins in terms of their amino acid and encoding DNA sequences. Such information would provide for the large scale production of the proteins, allow for identification of cells which express the phosphatases naturally and permit production of antibodies specifically reactive with the phosphatases. Moreover, elucidation of the substrates, regulatory mechanisms, and subcellular localization of these PTPs would contribute to an understanding of normal cell growth and provide information essential for the development of therapeutic agents useful for intervention in abnormal and/or malignant cell growth.

BRIEF DESCRIPTION OF THE INVENTION

As employed herein with respect to a protein tyrosine phosphatase, "density enhanced" denotes upregulated cellular mRNA transcription or translation and/or total cellular activity as a function of increased contact with neighboring cells.

In one aspect, the present invention provides purified and isolated polynucleotides (e.g., DNA and RNA transcripts, both sense and anti-sense strands) encoding a Type III density enhanced protein tyrosine phosphatase enzymatic activity exemplified by the human phosphatase huDEP-1 and variants, including fragments, thereof (i.e., fragments and deletion, addition or substitution analogs) which possess binding and/or immunological properties inherent to Type III density enhanced phosphatases. Preferred DNA molecules of the invention include cDNA, genomic DNA and wholly or partially chemically synthesized DNA molecules. A presently preferred polynucleotide is the DNA as set forth in SEQ ID NO: 1, encoding the human DEP-1 polypeptide of SEQ ID NO: 2. Also provided are recombinant plasmid and viral DNA constructions (expression constructs) which include Type III density enhanced phosphatase encoding sequences, especially constructions wherein the Type III density enhanced phosphatase encoding sequence is operatively linked to a homologous or heterologous transcriptional regulatory element or elements.

As another aspect of the invention, prokaryotic or eukaryotic host cells transformed or transfected with DNA sequences of the invention are provided which express a Type III density enhanced phosphatase polypeptide or variants thereof. Host cells of the invention are particularly useful for large scale production of Type III density enhanced phosphatase polypeptides, which can be isolated from either the host cell itself or the medium in which the host cell is grown. Host cells which express Type III density enhanced phosphatase polypeptides on the extracellular membrane surface are also useful as immunogens in the production of anti-Type III density enhanced phosphatase antibodies.

- 8 -

Also provided by the present invention are purified and isolated Type III density enhanced phosphatase polypeptides, including fragments and variants thereof. A preferred Type III density enhanced phosphatase polypeptide is set forth in SEQ ID NO: 2. Novel Type III density enhanced phosphatase 5 polypeptides and variant polypeptides may be obtained as isolates from natural sources, but are preferably produced by recombinant procedures involving host cells of the invention. Completely glycosylated, partially glycosylated and wholly un-glycosylated forms of the Type III density enhanced phosphatase polypeptide may be generated by varying the host cell selected for recombinant production 10 and/or post-isolation processing. Variant Type III density enhanced phosphatase polypeptides of the invention may comprise water soluble and insoluble polypeptides including analogs wherein one or more of the amino acids are deleted or replaced: (1) without loss, and preferably with enhancement, of one or more biological activities or immunological characteristics specific for Type III 15 density enhanced phosphatases; or (2) with specific disablement of a particular ligand/receptor binding or signalling function.

Also comprehended by the present invention are peptides, polypeptides, and other non-peptide molecules which specifically bind to Type III density enhanced phosphatases of the invention. Preferred binding molecules 20 include antibodies (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, anti-idiotype antibodies, CDR-grafted antibodies and the like), counterreceptors (e.g., membrane-associated and soluble forms) and other ligands (e.g., naturally occurring or synthetic molecules), including those which competitively bind Type III density enhanced phosphatases in the presence 25 of anti-Type III density enhanced phosphatase monoclonal antibodies and/or specific counterreceptors. Binding molecules are useful for purification of Type III density enhanced phosphatase polypeptides of the invention and for identifying cell types which express the polypeptide. Binding molecules are also useful for

- 9 -

modulating (*i.e.*, inhibiting, blocking or stimulating) the *in vivo* binding and/or signal transduction activities of Type III density enhanced phosphatases.

Hybridoma cell lines which produce antibodies specific for Type III density enhanced phosphatases are also comprehended by the invention.

5 Techniques for producing hybridomas which secrete monoclonal antibodies are well known in the art. Hybridoma cell lines may be generated after immunizing an animal with a purified Type III density enhanced phosphatase, or variants thereof, or cells which express a Type III density enhanced phosphatase or a variant thereof on the extracellular membrane surface. Immunogen cell types 10 include cells which express a Type III density enhanced phosphatase *in vivo*, or transfected or transformed prokaryotic or eukaryotic host cells which normally do not express the protein *in vivo*.

The value of the information contributed through the disclosure of 15 the DNA and amino acid sequences of human DEP-1 is manifest. In one series of examples, the disclosed human DEP-1 cDNA sequence makes possible the isolation of the human DEP-1 genomic DNA sequence, including transcriptional control elements. Transcriptional control elements comprehended by the invention include, for example, promoter elements and enhancer elements, as well as 20 elements which contribute to repression, or downregulation, of mRNA transcription. Control elements of this type may be 5' DNA sequences or 3' DNA sequences with respect to the protein-encoding structural gene sequences, and/or DNA sequences located in introns. The 5' and/or 3' control elements 25 may be proximal and/or distal the protein-encoding sequences of the structural gene. Identification of DNA sequences which modulate mRNA transcription in turn permits the identification of agents which are capable of effecting transcriptional modulation.

In another aspect, identification of polynucleotides encoding other Type III density enhanced phosphatases, huDEP-1 allelic variants and heterologous species (*e.g.*, rat or mouse) DNAs is also comprehended. Isolation

- 10 -

of the huDEP-1 genomic DNA and heterologous species DNAs may be accomplished by standard nucleic acid hybridization techniques, under appropriately stringent conditions, using all or part of the DEP-1 DNA or RNA sequence as a probe to screen an appropriate library. Alternatively, polymerase 5 chain reaction (PCR) using oligonucleotide primers that are designed based on the known nucleotide sequence can be used to amplify and identify other cDNA and genomic DNA sequences. Synthetic DNAs encoding Type III density enhanced phosphatase polypeptide, including fragments and other variants thereof, may be synthesized by conventional methods.

10 DNA sequence information of the invention also makes possible the development, by homologous recombination or "knockout" strategies [see, e.g., Capecchi, *Science* 244:1288-1292 (1989)], of rodents that fail to express a functional Type III density enhanced phosphatase polypeptide or that express a variant Type III density enhanced phosphatase polypeptide. Such rodents are 15 useful as models for studying the activities of Type III density enhanced phosphatases and modulators thereof *in vivo*.

20 DNA and amino acid sequences of the invention also make possible the analysis of Type III density enhanced phosphatase regions which actively participate in counterreceptor binding, as well as sequences which may regulate, rather than actively participate in, binding. Identification of motifs which 25 participate in transmembrane signal transduction is also comprehended by the invention. Also comprehended is identification of motifs which determine subcellular localization of the immature and mature Type III density enhanced phosphatase proteins.

25 DNA of the invention is also useful for the detection of cell types which express Type III density enhanced phosphatase polypeptides. Identification of such cell types may have significant ramifications for development of therapeutic and prophylactic agents. Standard nucleic acid hybridization techniques which utilize e.g., huDEP-1 DNA to detect corresponding RNAs, may

- 11 -

be used to determine the constitutive level of Type III density enhanced phosphatase transcription within a cell as well as changes in the level of transcription in response to internal or external agents. Identification of agents which modify transcription, translation, and/or activity of Type III density enhanced phosphatases can, in turn, be assessed for potential therapeutic or prophylactic value. DNA of the invention also makes possible *in situ* hybridization of *e.g.*, huDEP-1 DNA to cellular RNA, to determine the cellular localization of Type III density enhanced phosphatase specific messages within complex cell populations and tissues.

10 Polynucleotides of the present invention also provide a method whereby substrate or other molecules which interact with Type III density enhanced phosphatases can be identified. A presently preferred method for identifying interacting molecules comprises the steps of: a) transforming or transfecting appropriate host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA-binding domain and an activating domain; b) an optional step of cotransforming or co-transfected the same host cells with a protein tyrosine kinase (e.g., v-src, c-src or the like) in order to phosphorylate potential interacting components and/or substrates introduced as in (d) below; c) expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of *e.g.*, a huDEP-1 isoform and either the DNA-binding domain or the activating domain of the transcription factor; d) expressing in the host cells a library of second hybrid DNA sequences encoding second fusions of part or all of putative DEP-1 isoform-binding proteins and either the activating domain or DNA binding domain of the transcription factor which is not incorporated in the first fusion; e) detecting binding of DEP-1 isoform-binding proteins to the DEP-1 isoform in a particular host cell by detecting the production of reporter gene product in the host cell; and f) isolating second hybrid DNA sequences encoding DEP-1 isoform-binding protein from the particular host cell. Variations of the method altering

- 12 -

the order in which *e.g.*, the huDEP-1 isoforms and putative huDEP-1 isoform-binding proteins are fused to transcription factor domains, either at the amino terminal or carboxy terminal end of the transcription factor domains, are contemplated. In a preferred method, the promoter is the ADHI promoter, the 5 DNA-binding domain is the *lexA* DNA-binding domain, the activating domain is the GAL4 transactivation domain, the reporter gene is the *lacZ* gene and the host cell is a yeast host cell. Those of ordinary skill in the art will readily envision that any of a number of other reporter genes and host cells are easily amenable to this technique. Likewise, any of a number of transcription factors with distinct 10 DNA binding and activating domains can be utilized in this procedure, either with both the DNA binding and activating domains derived from the same transcription factor, or from different, but compatible transcription factors. As another variation of this method, mutant DEP-1 polypeptides, wherein a cysteine residue 15 in the catalytic domain has been substituted with a serine residue, can be employed in this technique. Mutations of this type have been demonstrated with other phosphatases to recognize and bind substrates, but do not dephosphorylate the substrate since the phosphatase is inactive as a result of the mutation.

An alternative identification method contemplated by the invention for detecting proteins which bind to a Type III density enhanced phosphatase 20 isoform comprises the steps of: a) transforming or transfecting appropriate host cells with a hybrid DNA sequence encoding a fusion between a putative Type III density enhanced phosphatase isoform-binding protein and a ligand capable of high affinity binding to a specific counterreceptor; b) expressing the hybrid DNA sequence in the host cells under appropriate conditions; c) immobilizing fusion 25 protein expressed by the host cells by exposing the fusion protein to the specific counterreceptor in immobilized form; d) contacting a Type III density enhanced phosphatase isoform with the immobilized fusion protein; and e) detecting the Type III density enhanced phosphatase isoform bound to the fusion protein using a reagent specific for the Type III density enhanced phosphatase isoform.

- 13 -

Presently preferred ligands/counterreceptor combinations for practice of the method are glutathione-S-transferase/glutathione, hemagglutinin/hemagglutinin-specific antibody, polyhistidine/nickel and maltose-binding protein/amylose.

Additional methods to identify proteins which specifically interact
5 with Type III density enhanced phosphatase (*i.e.*, substrates, ligands, modulators, *etc.*) are also contemplated by the invention. In one example, purified and isolated Type III density enhanced phosphatase polypeptide (*e.g.*, huDEP-1 polypeptide) can be covalently coupled to an immobilized support (*i.e.*, column resins, beads, *etc.*) and incubated with cell lysates to permit protein/protein
10 interactions. Proteins which interact with the immobilized DEP-1 polypeptide can then be eluted from the support with gradient washing techniques which are standard in the art.

As another example, protein overlay techniques can be employed. DNA from cells which either express *e.g.*, huDEP-1 or express polypeptides
15 which can modulate or bind to huDEP-1, can be isolated and a library constructed by standard methods. This library can then be expressed in a heterologous cell line and resulting colonies transferred to an immobilizing support. Expressed proteins from these colonies are then contacted with DEP-1 and incubated under appropriate conditions to permit DEP-1/protein interactions.
20 The resulting Type III density enhanced phosphatase/protein complexes formed can be detected by incubation with a specific Type III density enhanced phosphatase antibody. Colonies which interact with the specific antibody contain DNA encoding a protein which interacts with the Type III density enhanced phosphatase. Alternatively, cell or tissue lysates may be employed in this
25 technique, using cells or tissues which normally express DEP-1, or cells which have been previously transfected or transformed with DEP-1 encoding DNA.

- 14 -

BRIEF DESCRIPTION OF THE DRAWING

Numerous other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description thereof, reference being made to the drawing wherein:

5 Figures 1A through 1B are photographs of Northern blot analysis autoradiograms; and

Figures 2 shows the density-dependent expression of DEP-1.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is illustrated by the following examples relating to the isolation and characterization of genes encoding Type III density enhanced phosphatase polypeptides. Example 1 relates to the isolation of cDNA encoding human DEP-1. Example 2 discusses the tissue distribution of huDEP-1 as determined by Northern blot analysis. Example 3 addresses the generation of antibodies specific for DEP-1 and fragments thereof. Example 4 demonstrates expression of a huDEP-1 cDNA clone in COS cells. Example 5 relates to detection of endogenous expression of huDEP-1 in fibroblast cells. Example 6 addresses expression of huDEP-1 as a function of cell culture density. Example 7 relates to identification of ligands of huDEP-1. Example 8 discusses identification of modulators and substrates of huDEP-1 activity. Example 9 details characterization of the genomic huDEP-1 DNA.

Example 1

Isolation and Characterization of huDEP-1 cDNA

In initial efforts to isolate cDNA encoding a novel human phosphatase regulated by a cell density-dependent mechanism, PCR primers were synthesized based on conserved amino acid sequences common to many previously identified phosphatases. These primers were then used to amplify

- 15 -

polynucleotides from a cDNA library, the resulting amplification products were sequenced, and these sequences compared to previously reported DNA sequences.

5 Degenerate primers, corresponding to conserved PTP amino acid sequences set out in SEQ ID NO: 3 and SEQ ID NO: 4, were synthesized and used to prime a PCR with a HeLa cell cDNA library as template.

KCAQYWP SEQ ID NO: 3
HCSAGIG SEQ ID NO: 4

10 The corresponding primers used in the PCR reaction are set forth in SEQ ID NO: 5 and SEQ ID NO: 6, respectively, employing nucleotide symbols according to 37 U.S.C. § 1.882.

5'-AARTGYGCNCARTAYTGGCC-3' SEQ ID NO: 5
3'-GTRACRTCRGNCCITADCC-5' SEQ ID NO: 6

15 Sequencing of seventy-seven independent subclones revealed seven distinct sequences, six of which corresponded to PTPs for which DNA sequences had previously been published, and included PTP1B [Tonks, *et al.*, *J.Biol.Chem* 263:6722-6730 (1988)], TCPTP [Cool, *et al.*, *Proc.Natl.Acad.Sci.(USA)* 86:5257-5261 (1989)], RPTP α [Krueger, *et al.*, *EMBO J.* 9:3241-3252 (1990)], LAR [Streuli, *et al.*, *J.Exp.Med.* 168:1523-1530 (1988)], PTPH1 [Yang and Tonks, *Proc.Natl.Acad.Sci.(USA)* 88:5949-5953 (1991)], and PTP μ [Gebbink, *et al.*, *FEBS Lett.* 290:123-130 (1991)]. The seventh clone was determined to comprise 20 a unique 300 bp PCR fragment and was used to screen an oligo-dT-primed HeLa cell cDNA library (Stratagene, La Jolla, CA) in an effort to isolate a corresponding full-length cDNA. Approximately 1.8×10^6 phage plaques were screened as previously described [Yang and Tonks, *Proc.Natl.Acad.Sci.(USA)* 88:5949-5953 (1991)] and twenty-four positive clones were identified. The 25 largest insert, a 5.1 kb cDNA, was cloned into pUC119, sequenced by the dideoxy chain termination method, and found to contain an open reading frame of 4011 nucleotides encoding a novel receptor-like PTP of 1337 amino acids. The DNA sequence of the 5.1 kb insert is set out in SEQ ID NO: 1, and its predicted amino

- 16 -

acid sequence is set out in SEQ ID NO: 2. This human density-enhanced PTP was designated huDEP-1.

The proposed initiating ATG codon of the huDEP-1 gene is flanked by a purine (G) at the -3 position and is thus in agreement with the Kozak rules for initiation [Kozak, *J. Cell Biol.* 108:229-241 (1989)]. There is an in-frame stop codon approximately 290 bp upstream of the predicted initiation site, and the initiating ATG is followed by a hydrophobic region that may serve as a signal sequence. Based on the statistical analysis of known cleavage sites for the signal peptidase [von Heijne, *Nuc. Acids Res.* 14:4683-4690 (1986)], the amino terminus of the mature huDEP-1 polypeptide is assigned to Gly³⁷. A second hydrophobic region is found between amino acids 977 and 996, and is followed by a stretch of predominantly basic residues, characteristic of a stop transfer sequence. Therefore, an extracellular region of 940 amino acids and an intracellular portion of 341 amino acids are predicted for the mature huDEP-1 protein. The extracellular domain comprises eight FNIII domains, and thirty-three potential sites for N-linked glycosylation are predicted. Thus, huDEP-1 conforms to the RPTP Type III topography according to the nomenclature of Fischer *et al.*, *supra*. Unlike most RPTPs which possess a tandem repeat of catalytic domains, the cytoplasmic region contains a single catalytic domain spanning amino acid residues 1060 through 1296. Human DEP-1 is therefore representative of an expanding group of RPTPs with a single catalytic domain that includes PTP β [Krueger, *et al.*, *EMBO J.* 9:3241-3252 (1990)], DPTPIOD of *Drosophila* [Tian, *et al.*, *Cell* 76:675-685 (1991); Yang, *et al.*, *Cell* 67:661-673 (1991)], DPTP4E of *Drosophila* [Oon, *et al.*, *J. Biol. Chem.* 268:23964-23971 (1993)], and the recently described SAP-1 enzyme [Matozaki, *et al.*, *J. Biol. Chem.* 269:2075-2081 (1994)]. Amino acid sequence comparison of the catalytic domain of huDEP-1 with other PTP domains revealed huDEP-1 is most closely related to PTP β and SAP-1. The sequence includes several Ser-Pro motifs, as well as potential sites for phosphorylation by casein kinase II.

- 17 -

Example 2

Northern Analysis of huDEP-1 Tissue Distribution

Because the expression of PTPs has previously been demonstrated to be ubiquitous in eukaryotes, various human tissues were analyzed in order to 5 determine the relative degree of huDEP-1 mRNA expression.

RNA Multi Tissue Northern blot filters (Clontech, Palo Alto, CA), containing immobilized RNA from various human tissues, were probed with a 1.6 kb *Hind*III/*Eco*RI fragment of the huDEP-1 cDNA previously radiolabeled to a specific activity of 1.5 X 10⁶ cpm/ng using a Megaprime DNA labeling kit 10 (Amersham, Arlington Heights, IL). This probe represented the entire length of the isolated huDEP-1 cDNA. Hybridization was performed for 16 hours at 65°C in a hybridization buffer containing 0.5 M Na₂HPO₄, 7% SDS, 1 mM EDTA, and labeled probe at a concentration of 10⁶ cpm/ml. Filters were then washed 5 times 15 at 65°C in 40 mM Na₂HPO₄, 1% SDS, and 1 mM EDTA. The membrane was then subjected to autoradiography. The results are presented in Figures 1A and 1B, wherein the human tissue source of immobilized RNA is as follows. In Figure 1A, RNA in lane 2 is from heart, lane 3 from brain, lane 4 from placenta, lane 5 from lung, lane 6 from liver, lane 7 from skeletal muscle, lane 8 from kidney, and lane 9 from pancreas. In Figure 1B, RNA in lane 2 is from spleen, 20 lane 3 from thymus, lane 4 from prostate, lane 5 from testis, lane 6 from ovary, lane 7 from small intestines, lane 8 from colon, and lane 9 from peripheral blood leukocyte.

25 Northern analysis indicated that huDEP-1 is expressed in most tissues analyzed, with particularly high mRNA levels detected in placenta, kidney, spleen and peripheral blood leukocytes.

- 18 -

Example 3

Generation of huDEP-1 Polyclonal Antibodies

Two peptides, unique to huDEP-1 and corresponding to amino acid residues 1297 through 1315 and residues 1321 through 1334 in SEQ ID NO: 2 (downstream from the catalytic region) were synthesized with an additional amino terminal cysteine residue and conjugated to rabbit serum albumin (RSA) with *m*-maleimidobenzoic acid N-hydroxysuccinimide ester (MBS)(Pierce, Rockford, IL). Immunization protocols with these peptides were performed by Cocalico Biologicals (Reamstown, PA). Initially, a pre-bleed of the rabbits was performed prior to immunization. The first immunization included Freund's complete adjuvant and 500 μ g conjugated peptide or 100 μ g purified peptide. All subsequent immunizations, performed four weeks after the previous injection, included Freund's incomplete adjuvant with the same amount of protein. Bleeds were conducted seven to ten days after the immunizations.

For affinity purification of the antibodies, huDEP-1 peptide conjugated to RSA with MBS, was coupled to CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden). Antiserum was diluted 10-fold in 10 mM Tris-HCl, pH 7.5, and incubated overnight with the affinity matrix. After washing, bound antibodies were eluted from the resin with 100 mM glycine, pH 2.5.

The antibody generated against conjugated amino acid residues 1297 through 1315 was designated anti-CSH-241, and the antibody raised against the conjugated peptide corresponding to amino acid residues 1321 through 1334 was designated anti-CSH-243.

- 19 -

Example 4

Expression of huDEP-1 by Transfected Host Cells

To study the protein product of the huDEP-1 cDNA, the 5.1 kb *Eco*RI insert was cloned into the expression vector pMT2 [Sambrook, *et al.*, 5 Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989) pp 16.17-16.22] and transfected into COS cells grown in DMEM supplemented with 10% FCS. Transfections were performed employing calcium phosphate techniques [Sambrook, *et al* (1989) pp. 16.32-16.40, *supra*] and cell lysates were prepared forty-eight hours after transfection from both transfected 10 and untransfected COS cells. Lysates were subjected to analysis by immunoblotting using anti-CSH-243 antibody, and PTP assays of immune complexes as addressed below.

In immunoblotting experiments, preparation of cell lysates and electrophoresis were performed. Protein concentration was determined using 15 BioRad protein assay solutions. After semi-dry electrophoretic transfer to nitro-cellulose, the membranes were blocked in 500 mM NaCl, 20 mM Tris, pH 7.5, 0.05% Tween-20 (TTBS) with 5% dry milk. After washing in TTBS and incubation with secondary antibodies (Amersham), enhanced chemiluminescence (ECL) protocols (Amersham) were performed as described by the manufacturer 20 to facilitate detection.

For immune complex PTP assays, 60 μ g of cell lysate were immunoprecipitated with 20 μ l of anti-CSH-243 antisera or preimmune rabbit serum bound to 25 μ l of Protein-A Sepharose (Pharmacia). After overnight 25 incubation at 4° C, the immune complexes were washed three times in washing buffer (1% Triton X-100, 150 mM NaCl, 20 mM Hepes, pH 7.5, 5 μ g/ml aprotinin, 5 μ g/ml leupeptin, 1 mM benzamidine, and 1 mM DTT) and once in assay buffer (25 mM imidazole, pH 7.2, 0.5 mg/ml BSA, and 1 mM DTT). Protein-A Sepharose immune complexes were then resuspended in 150 μ l of assay buffer and assayed for PTP activity as triplicates. Assays were performed for 6

- 20 -

minutes at 30° C in a total volume of 60 μ l using 3 μ M [32 P-Tyr]-reduced carboxymethylated (RCM) lysozyme as substrate [Flint, *et al.*, *EMBO J.* 12:1937-1946 (1993)].

5 Affinity-purified anti-CSH-243 antibodies specifically detected a protein of 180 kD molecular weight in lysates from transfected cells. Furthermore, when immune complexes were analyzed for PTP activity, almost 10-fold higher activity was detected in anti-CSH-243 immune complexes from the transfected cells compared to the untransfected cells. This PTP activity was largely absent in immune complexes derived from immunoprecipitations with 10 blocked antiserum or preimmune serum. It was concluded that the huDEP-1 cDNA encodes a 180 kD protein with intrinsic PTP activity.

Example 5

Endogenous Expression of huDEP-1

15 To characterize endogenously expressed huDEP-1, lysates from different cell lines including CEM (ATCC CCL 119), HeLa (ATCC CCL 2), 293 (ATCC CRL 1573), Jurkat (ATCC TIB 152), K562 (ATCC CCL243), HL60 (ATCC CCL 240), WI38 (ATCC CCL 75) and AG 1518 (Coriell Cell Repositories, Camden, NJ) were analyzed by immunoblotting with antibody anti-CSH-243 as described in Example 4.

20 WI38 cells, a diploid fetal lung fibroblast-like cell line with finite life span, showed the highest expression. Similar levels of expression were also detected in AG 1518 foreskin fibroblast cells.

25 To further examine the expression of huDEP-1, lysates from metabolically labeled cells were analyzed by immunoprecipitation and SDS-gel electrophoresis. Confluent cultures of WI38 and AG 1518 cells were metabolically labeled for four hours in methionine-free DMEM supplemented with 1 mg/ml bovine serum albumin (BSA) and 0.15 mCi/ml Translabel (ICN, Costa Mesa, CA). Cells were lysed in 0.5% DOC, 0.5% Triton X-100, 150 mM NaCl,

- 21 -

20 mM Hepes, pH 7.5, 5 μ g/ml aprotinin, 5 μ g/ml leupeptin, 1 mM benzamidine, 1 mM DTT (lysis buffer) and lysates were centrifuged at 15,000 x g for 15 minutes. Lysates corresponding to approximately 2 x 10⁶ cells were then incubated with 20 μ l of anti-CSH-243 or anti-CSH-243. After incubation for four 5 hours at 4° C, 50 μ l of a 1:1 Protein-A-Sepharose slurry was added to bind the protein/antibody complexes and incubation continued for 60 minutes. Immune complexes adsorbed to the Protein-A-Sepharose were collected by centrifugation and washed three times in 1% Triton X-100, 150 mM NaCl, 20 mM Hepes, pH 10 7.5, 5 μ g/ml aprotinin, 5 μ g/ml leupeptin, 1 mM benzamidine, 1 mM DTT (washing buffer) and once in 20 mM Tris, pH 7.5. Samples were eluted from the resin by incubation at 95° C for 3 minutes in reducing SDS-sample buffer and analyzed by SDS-gel electrophoresis on 7% gels, followed by fluorography.

In both WI38 and AG 1518 cells, a protein of 180 kD was 15 recognized specifically by the unblocked antisera. Anti-CSH-243 antisera immunoprecipitation with WI38 cell lysate also yielded significantly higher amounts (approximately 10 to 20 fold higher) of activity than precipitations with pre-immune serum or antiserum that had been previously incubated with 200 μ g/ml of peptide-conjugate.

It appears that huDEP-1 is a phosphoprotein *in vivo* because the 20 fact that the anti-CSH-243 antibody was capable of immunoprecipitating a 180 kD [³²P]-labeled protein from a cell lysate of WI38 cells which had been metabolically labelled with [³²P]-inorganic phosphate.

Example 6

Cell Density-Dependent Expression and Activity of huDEP-1.

25 WI38 cell lysates from sparse (less than 7,000 cells /cm²) or dense (more than 25,000 cells/cm²) cultures were compared for levels of expressed huDEP-1 protein by immunoblotting with anti-CSH-243 antibody as described in Example 4. A dramatic, ten- to twenty-fold increase in huDEP-1 expression was

- 22 -

detected in dense cell cultures as shown in Figure 2. Since 3 μ g of total cell lysate from more confluent culture gave a relatively strong signal, and 15 μ g of lysates from sparse cultures were below detection, it was estimated that at least 10-fold higher amounts of huDEP-1 are present in cells from dense cultures.

5 Similar results were obtained with anti-CSH-241. When the amounts of PTP1B in cell lysates from sparse and dense cells were compared using an anti-PTP1B monoclonal antibody FG6 (Oncogene Science, Uniondale, NY), no difference was observed. The observed effects on huDEP-1 expression are not restricted to WI38 cells as similar results were obtained in AG 1518 cells.

10 In order to determine if enzyme activity was also regulated by a density-dependent mechanism, huDEP-1 and PTP1B immune complexes and total cell lysates from both sparse and dense WI38 and AG 1518 cell cultures were also analyzed for phosphatase activity using the PTP assay. For immune complex PTP assays, 60 μ g of cell lysate were immunoprecipitated with 20 μ l of anti-CSH-243 15 antisera (with or without pretreatment with antigen) or preimmune serum bound to 25 μ l of Protein-A Sepharose. After incubation overnight at 4° C, immune complexes were washed three times in washing buffer and once in 25 mM imidazole, pH 7.2, 0.5 mg/ml BSA, 1 mM DTT (assay buffer). Protein-A-Sepharose immune complexes were then suspended in 150 μ l of assay 20 buffer and assayed for PTP activity as triplicates. Assays were performed for 6 minutes at 30° C in a total volume of 60 μ l using 3 μ M [32 P-Tyr] RCM lysozyme as substrate [Flint, *et al.*, *supra*].

25 In agreement with the increased huDEP-1 protein expression demonstrated in the immunoblotting experiments, huDEP-1 enzyme activity also increased in the dense cell cultures. The observed increase in activity in huDEP-1/CSH-243 immunoprecipitates from dense cultures (approximately two- to three-fold) was not as great as the observed increase in protein expression in dense cultures, most likely due to incomplete precipitation of all of the PTP using

- 23 -

anti-CSH-243 antisera. No difference was observed in activity of PTP1B/FG6 immunoprecipitates or total cell lysates from sparse and dense cell cultures.

Finally, to investigate the kinetics of the density-dependent upregulation of huDEP-1 expression, lysates of WI38 and AG 1518 cells at intermediate cell densities were included in the immunoblotting analysis. The highest expression was found in cells at saturation density, however, at intermediate densities an increase in expression with respect to sparse cell cultures was also observed. Thus, the upregulation of huDEP-1 expression appears to be initiated prior to saturation density and not a result of growth arrest.

While the precise mechanism by which huDEP-1 expression is induced remains unclear, the demonstration that expression was induced in two distinct cell lines as cells approach confluence suggests involvement of huDEP-1 in promoting net dephosphorylation of proteins, countering the effects of growth promoting PTK activity. This possibility, in combination with the broad distribution of huDEP-1 expression, suggests that huDEP-1 may be involved in a general mechanism for contact inhibition of cell growth.

Example 7

Identification of DEP-1 Ligands

The possibility that DEP-1 functions as an adhesion molecule will be tested using the Sf9 cell system [Brady-Kalnay, *et al.*, *J. Cell Biol.* 122:961-972 (1993)] following transfection with DEP-1 cDNA. In addition to studies following transient expression, stable cell lines overexpressing DEP-1 will be generated.

If DEP-1 functions as an adhesion molecule, the extracellular counterreceptor(s) will be identified. One possibility is that, like PTP μ , DEP-1 binding is homophilic, where one DEP-1 molecule binds another DEP-1 molecule on an adjacent cell. Alternatively, DEP-1 specifically recognize a non-DEP-1 molecule in a heterophilic binding mechanism.

- 24 -

In addition, a number of deletion and site-directed mutagenesis strategies well known in the art will be applied to identify the important segments in the protein that confer binding specificity. Analysis of 2D gels of proteins that react with anti-phosphotyrosine antibodies, for example monoclonal antibody 5 4G10 (UBI, Lake Placid, NY), will be used to initiate studies as to the effect on activity of engagement of the extracellular segment of the PTP in either homophilic binding interactions or antibody binding.

Use of "epitope" library technology [Scott and Smith, *Science* 10 249:386-390 (1990)] will be employed to identify peptide sequences that interact with DEP-1. This approach will prove particularly useful in the search for ligands for DEP-1 whose extracellular segment, comprising multiple FNIII repeats, may bind low M_r factors.

Protein:protein interactions have previously been reported for FNIII 15 sequences and specific binding proteins, and this information will be utilized in several approaches to identify proteins which specifically interact with the extracellular domain of DEP-1. Specifically, protein:protein interactions will be investigated in cell "panning" experiments [Seed and Aruffo, *Proc. Natl. Acad. Sci. (USA)* 84:3365-3369 (1987)], gel overlay assays [Hirsch, *et al.*, *J. Biol. Chem.* 267:2131-2134 (1992); Carr and Scott, *Trends in Biochemical Sci.* 20 17:246-249 (1992)], band shift analysis [Carr, *et al.*, *J. Biol. Chem.* 267:13376-13382 (1992)], affinity chromatography, screening of expression libraries [Young and Davis, *Proc. Natl. Acad. Sci. (USA)* 80:1194-1198 (1983)], etc.

- 25 -

Example 8

Identification of Modulators/Substrates of DEP-1

Potential substrates of predicted physiological relevance will be tested for activity against the catalytic domain *in vitro*.

5 In addition, yeast screening systems [Fields and Song, *Nature* 340:245-246 (1989); Yang, *et al.*, *Science* 257:6810682 (1992); Vojtek, *et al.*, *Cell* 74:205-214 (1993)] will be utilized, particularly with reference to co-expression with a protein tyrosine kinase, for example, v-src or c-src, to isolate proteins with the capacity to regulate DEP-1 activity.

10 In a further attempt to identify substrates for DEP-1, a mutant form in which the cysteinyl residues of the active center has been replaced by serine will be expressed. Recent studies suggest that substrates bind to and remain complexed with the inactive phosphatase. The mutant PTP is capable of binding substrate molecules but traps them in a "dead end" complex that can be isolated 15 by standard immunoprecipitation techniques [Sun, *et al.*, *Cell* 75:487-493 (1993)]. Potential substrates may be co-immunoprecipitated with the mutant PTP from 35 S-labeled cells. Alternatively, wild-type, or native, DEP-1 enzyme may be utilized in this technique. Initial studies in this direction may make use of chimeric molecules, for which antibodies to the extracellular growth factor binding segment 20 are commercially available, while antibodies are raised to the bona fide DEP-1 sequences.

Example 9

Characterization of the Genomic DEP-1 Gene

25 Isolation of the cDNA sequences for DEP-1 will permit the isolation and purification of the corresponding genomic sequences for DEP-1. In preliminary work, it has been demonstrated that huDEP-1 mapped to human chromosome 11p, band 11.2 or the interface of 11.2 and 11.3. Isolation of these genomic DEP-1 sequences will permit the identification of putative regulatory

- 26 -

sequences for DEP-1 transcription, and presumably identification of *trans*-acting transcriptional modulators of DEP-1 expression. In addition, isolation and purification of the human genomic clone will permit screening of libraries in other species to determine if homologous counterparts exist in the species.

5 Identification of a homologous counterpart in mice will be of particular importance because of the possibility of generating a knockout strain. Mouse strains which do not express a particular protein are of considerable importance in that they permit determination of indications associated with absence of the protein in a living animal.

10 While the present invention has been described in terms of specific methods and compositions, it is understood that variations and modifications will occur to those skilled in the art. Therefore, only such limitations as appear in the claims should be placed on the invention.

- 27 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANTS: Tonks, Nicholas K. and Östman, Arne
- (ii) TITLE OF INVENTION: Density Enhanced Protein Tyrosine Phosphatase
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
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 - (E) COUNTRY: United States of America
 - (F) ZIP: 60606
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Borun, Michael F.
 - (B) REGISTRATION NUMBER: 25,447
 - (C) REFERENCE/DOCKET NUMBER: 27866/31954
- (ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5117 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 350..4364

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCCCAGCCGC ATGACGCGCG GAGGAGGCAG CGGGACGAGC GCGGGAGCCG GGACCGGGTA	60
GCCGCGCGCT GGGGGTGGGC GCCGCTCGCT CCGCCCCGCG AAGCCCCCTGC GCGCTCAGGG	120

- 28 -

ACCGCGGCCCG	CCCGCGGCAG	CCGCGCTAGG	CTCCGGCGTG	TGGCCGCGGC	CGCCGCCGCG	180
CTGCCATGTC	TCCGGGGAAAG	CCGGGGCGGG	CGGAGCGGGG	ACGAGGCGGA	CCGGCTGGCG	240
GAGGAGGAGG	CGAAGGAGAC	GGCAGGAGGC	GGCGACGACG	GTGCCCCGGC	TGGGGCCAC	300
GGCGGGGCC	GATTGCGCG	TCCGGGGCAC	GTTCAGGGC	GCGCGGGC	ATG AAG	355
					Met Lys	
					1	
CCG GCG GCG	CGG GAG GCG	CGG CTG CCT	CCG CGC TCG	CCC GGG CTG	CGC	403
Pro Ala Ala	Arg Glu Ala	Arg Leu Pro	Pro Arg Ser	Pro Gly	Leu Arg	
5	10	15				
TGG GCG CTG CCG	CTG CTG CTG CTG	CTG CGC CTG	GGC CAG ATC	CTG		451
Trp Ala Leu Pro	Leu Leu Leu Leu	Leu Leu Leu	Arg Leu Gly	Gln Ile	Leu	
20	25	30				
TGC GCA GGT GGC	ACC CCT AGT CCA	ATT CCT GAC	CCT TCA GTC	GCA ACT		499
Cys Ala Gly	Gly Thr Pro Ser	Pro Ile Pro	Asp Pro Ser	Val Ala Thr		
35	40	45	50			
GTT GCC ACA GGG	GAA AAT GGC ATA	ACG CAG ATC	AGC AGT ACA	GCA GAA		547
Val Ala Thr	Gly Glu Asn	Gly Ile Thr	Gln Ile Ser	Ser Thr Ala	Glu	
55	60	65				
TCC TTT CAT AAA	CAG AAT GGA ACT	GGA ACA CCT	CAG GTG GAA	ACA AAC		595
Ser Phe His	Lys Gln Asn	Gly Thr Gly	Thr Pro Gln	Val Glu	Thr Asn	
70	75	80				
ACC ACT GAG GAT	GGT GAA AGC TCT	GGA GCC AAC	GAT AGT TTA	AGA ACA		643
Thr Ser Glu Asp	Gly Glu Ser Ser	Gly Ala Asn	Asp Ser	Leu Arg	Thr	
85	90	95				
CCT GAA CAA GGA	TCT AAT GGG ACT	GAT GGG GCA	TCT CAA AAA	ACT CCC		691
Pro Glu Gln	Gly Ser Asn	Gly Thr Asp	Gly Ala Ser	Gln Lys	Thr Pro	
100	105	110				
AGT AGC ACT GGG	CCC AGT CCT GTG	TTT GAC ATT AAA	GCT GTT TCC	ATC		739
Ser Ser Thr	Gly Pro Ser	Pro Val Phe	Asp Ile Lys	Ala Val Ser	Ile	
115	120	125	130			
AGT CCA ACC AAT	GTG ATC TTA ACT	TGG AAA AGT	AAT GAC	ACA GCT GCT		787
Ser Pro Thr Asn	Val Ile Leu Thr	Trp Lys Ser	Asn Asp	Thr Ala Ala		
135	140	145				
TCT GAG TAC AAG	TAT GTA GAA	CAT AAG ATG	GAA AAT GAG	AAG ACA		835
Ser Glu Tyr	Lys Tyr Val	Val Lys His	Lys Met	Glu Asn	Glu Lys Thr	
150	155	160				
ATT ACT GTT GTG	CAT CAA CCA	TGG TGT AAC	ATC ACA	GGC TTA CGT	CCA	883
Ile Thr Val Val	His Gln Pro	Trp Cys Asn	Ile Thr	Gly Leu	Arg Pro	
165	170	175				
GCG ACT TCA TAT	GTA TTC TCC ATC	ACT CCA GGA	ATA GGC AAT	GAG ACT		931
Ala Thr Ser	Tyr Val Phe	Ser Ile Thr	Pro Gly	Ile Gly	Asn Glu Thr	
180	185	190				
TGG GGA GAT CCC	AGA GTC ATA AAA	GTC ATC ACA	GAG CCG	ATC CCA	GTT	979
Trp Gly Asp Pro	Arg Val Ile Lys	Val Ile Thr	Glu Pro	Ile Pro	Val	
195	200	205	210			

- 29 -

TCT GAT CTC CGT GTT GCT CAC GGG TGT GAG GAA GGC TGC TCT CTC TCC	1027
Ser Asp Leu Arg Val Ala His Gly Cys Glu Glu Gly Cys Ser Leu Ser	
215 220 225	
TGG AGC AAT GGC AAT GGC ACC GCC TCC TGC CGG GTT CTT CTT GAA AGC	1075
Trp Ser Asn Gly Asn Gly Thr Ala Ser Cys Arg Val Leu Leu Glu Ser	
230 235 240	
ATT GGA AGC CAT GAG GAG TTG ACT CAA GAC TCA AGA CTT CAG GTC AAT	1123
Ile Gly Ser His Glu Glu Leu Thr Gln Asp Ser Arg Leu Gln Val Asn	
245 250 255	
ATC TCG GAC CTG AAG CCA GGG GTT CAA TAC AAC ATC AAC CCG TAT CTT	1171
Ile Ser Asp Leu Lys Pro Gly Val Gln Tyr Asn Ile Asn Pro Tyr Leu	
260 265 270	
CTA CAA TCA AAT AAG ACA AAG GGA GAC CCC TTG GCA CAG AAG GTG GCT	1219
Leu Gln Ser Asn Lys Thr Lys Gly Asp Pro Leu Ala Gln Lys Val Ala	
275 280 285 290	
TGG ATG CCA GCA ATA CAG AGA GAA GCC GGG CAG GGA GCC CCA CCG CCC	1267
Trp Met Pro Ala Ile Gln Arg Glu Ala Gly Gln Gly Ala Pro Pro Pro	
295 300 305	
CTG TGC ATG ATG AGT CCC TTC GTG GGA CCT GTG GAC CCA TCC TCC GGC	1315
Leu Cys Met Met Ser Pro Phe Val Gly Pro Val Asp Pro Ser Ser Gly	
310 315 320	
CAG CAG TCC CGA GAC ACG GAA GTC CTG CTT GTC GGG TTA GAG CCT GGC	1363
Gln Gln Ser Arg Asp Thr Glu Val Leu Leu Val Gly Leu Glu Pro Gly	
325 330 335	
ACC CGA TAC AAT GCC ACC GTT TAT TCC CAA GCA GCG AAT GGC ACA GAA	1411
Thr Arg Tyr Asn Ala Thr Val Tyr Ser Gln Ala Ala Asn Gly Thr Glu	
340 345 350	
GGA CAG CCC CAG GCC ATA GAG TTC AGG ACA AAT GCT ATT CAG GTT TTT	1459
Gly Gln Pro Gln Ala Ile Glu Phe Arg Thr Asn Ala Ile Gln Val Phe	
355 360 365 370	
GAC GTC ACC GCT GTG AAC ATC AGT GCC ACA AGC CTG ACC CTG ATC TGG	1507
Asp Val Thr Ala Val Asn Ile Ser Ala Thr Ser Leu Thr Leu Ile Trp	
375 380 385	
AAA GTC AGC GAT AAC GAG TCG TCA TCT AAC TAT ACC TAC AAG ATA CAT	1555
Lys Val Ser Asp Asn Glu Ser Ser Asn Tyr Thr Tyr Lys Ile His	
390 395 400	
GTG GCG GGG GAG ACA GAT TCT TCC AAT CTC AAC GTC AGT GAG CCT CGC	1603
Val Ala Gly Glu Thr Asp Ser Ser Asn Leu Asn Val Ser Glu Pro Arg	
405 410 415	
GCT GTC ATC CCC GGA CTC CGC TCC AGC ACC TTC TAC AAC ATC ACA GTG	1651
Ala Val Ile Pro Gly Leu Arg Ser Ser Thr Phe Tyr Asn Ile Thr Val	
420 425 430	
TGT CCT GTC CTA GGT GAC ATC GAG GGC ACG CCG GGC TTC CTC CAA GTG	1699
Cys Pro Val Leu Gly Asp Ile Glu Gly Thr Pro Gly Phe Leu Gln Val	
435 440 445 450	
CAC ACC CCC CCT GTT CCA GTT TCT GAC TTC CGA GTG ACA GTG GTC AGC	1747

- 30 -

His Thr Pro Pro Val Pro Val Ser Asp Phe Arg Val Thr Val Val Ser			
455	460	465	
ACG ACG GAG ATC GGC TTA GCA TGG AGC AGC CAT GAT GCA GAA TCA TTT			1795
Thr Thr Glu Ile Gly Leu Ala Trp Ser Ser His Asp Ala Glu Ser Phe			
470	475	480	
CAG ATG CAT ATC ACA CAG GAG GGA GCT GGC AAT TCT CGG GTA GAA ATA			1843
Gln Met His Ile Thr Gln Glu Gly Ala Gly Asn Ser Arg Val Glu Ile			
485	490	495	
ACC ACC AAC CAA AGT ATT ATC ATT GGT GGC TTG TTC CCT GGA ACC AAG			1891
Thr Thr Asn Gln Ser Ile Ile Gly Gly Leu Phe Pro Gly Thr Lys			
500	505	510	
TAT TGC TTT GAA ATA GTT CCA AAA GGA CCA AAT GGG ACT GAA GGG GCA			1939
Tyr Cys Phe Glu Ile Val Pro Lys Gly Pro Asn Gly Thr Glu Gly Ala			
515	520	525	530
TCT CGG ACA GTT TGC AAT AGA ACT GTT CCC AGT GCA GTG TTT GAC ATC			1987
Ser Arg Thr Val Cys Asn Arg Thr Val Pro Ser Ala Val Phe Asp Ile			
535	540	545	
CAC GTG GTC TAC GTC ACC ACC ACG GAG ATG TGG CTG GAC TGG AAG AGC			2035
His Val Val Tyr Val Thr Thr Glu Met Trp Leu Asp Trp Lys Ser			
550	555	560	
CCT GAC GGT GCT TCC GAG TAT GTC TAC CAT TTA GTC ATA GAG TCC AAG			2083
Pro Asp Gly Ala Ser Glu Tyr Val Tyr His Leu Val Ile Glu Ser Lys			
565	570	575	
CAT GGC TCT AAC CAC ACA AGC ACG TAT GAC AAA GCG ATT ACT CTC CAG			2131
His Gly Ser Asn His Thr Ser Thr Tyr Asp Lys Ala Ile Thr Leu Gln			
580	585	590	
GGC CTG ATT CCG GGC ACC TTA TAT AAC ATC ACC ATC TCT CCA GAA GTG			2179
Gly Leu Ile Pro Gly Thr Leu Tyr Asn Ile Thr Ile Ser Pro Glu Val			
595	600	605	610
GAC CAC GTC TGG GGG GAC CCC AAC TCC ACT GCA CAG TAC ACA CGG CCC			2227
Asp His Val Trp Gly Asp Pro Asn Ser Thr Ala Gln Tyr Thr Arg Pro			
615	620	625	
AGC AAT GTG TCC AAC ATT GAT GTA AGT ACC AAC ACC ACA GCA GCA ACT			2275
Ser Asn Val Ser Asn Ile Asp Val Ser Thr Asn Thr Ala Ala Thr			
630	635	640	
TTA AGT TGG CAG AAC TTT GAT GAC GCC TCT CCC ACG TAC TCC TAC TGC			2323
Leu Ser Trp Gln Asn Phe Asp Asp Ala Ser Pro Thr Tyr Ser Tyr Cys			
645	650	655	
CTT CTT ATT GAG AAG GCT GGA AAT TCC AGC AAC GCA ACA CAA GTA GTC			2371
Leu Leu Ile Glu Lys Ala Gly Asn Ser Ser Asn Ala Thr Gln Val Val			
660	665	670	
ACG GAC ATT GGA ATT ACT GAC GCT ACA GTC ACT GAA TTA ATA CCT GGC			2419
Thr Asp Ile Gly Ile Thr Asp Ala Thr Val Thr Glu Leu Ile Pro Gly			
675	680	685	690
TCA TCA TAC ACA GTG GAG CTC TTT GCA CAA GTA GGG GAT GGG ATC AAG			2467
Ser Ser Tyr Thr Val Glu Leu Phe Ala Gln Val Gly Asp Gly Ile Lys			

- 31 -

695	700	705	
TCA CTG GAA CCT GGC CGG AAG TCA TTC TGT ACA GAT CCT GCG TCC ATG Ser Leu Glu Pro Gly Arg Lys Ser Phe Cys Thr Asp Pro Ala Ser Met 710	715	720	2515
GCC TCC TTC GAC TGC GAA GTG GTC CCC AAA GAG CCA GCC CTG GTT CTC Ala Ser Phe Asp Cys Glu Val Val Pro Lys Glu Pro Ala Leu Val Leu 725	730	735	2563
AAA TGG ACC TGC CCT CCT GGC GCC AAT GCA GGC TTT GAG CTG GAG GTC Lys Trp Thr Cys Pro Pro Gly Ala Asn Ala Gly Phe Glu Leu Glu Val 740	745	750	2611
AGC AGT GGA GCC TGG AAC AAT GCG ACC CAC CTG GAG AGC TGC TCC TCT Ser Ser Gly Ala Trp Asn Asn Ala Thr His Leu Glu Ser Cys Ser Ser 755	760	765	2659
GAG AAT GGC ACT GAG TAT AGA ACG GAA GTC ACG TAT TTG AAT TTT TCT Glu Asn Gly Thr Glu Tyr Arg Thr Glu Val Thr Tyr Leu Asn Phe Ser 775	780	785	2707
ACC TCG TAC AAC ATC AGC ATC ACC ACT GTG TCC TGT GGA AAG ATG GCA Thr Ser Tyr Asn Ile Ser Ile Thr Thr Val Ser Cys Gly Lys Met Ala 790	795	800	2755
GCC CCC ACC CGG AAC ACC TGC ACT ACT GGC ATC ACA GAT CCC CCT CCT Ala Pro Thr Arg Asn Thr Cys Thr Thr Gly Ile Thr Asp Pro Pro Pro 805	810	815	2803
CCA GAT GGA TCC CCT AAT ATT ACA TCT GTC AGT CAC AAT TCA GTA AAG Pro Asp Gly Ser Pro Asn Ile Thr Ser Val Ser His Asn Ser Val Lys 820	825	830	2851
GTC AAG TTC AGT GGA TTT GAA GCC AGC CAC GGA CCC ATC AAA GCC TAT Val Lys Phe Ser Gly Phe Glu Ala Ser His Gly Pro Ile Lys Ala Tyr 835	840	845	2899
GCT GTC ATT CTC ACC ACC GGG GAA GCT GGT CAC CCT TCT GCA GAT GTC Ala Val Ile Leu Thr Thr Gly Glu Ala Gly His Pro Ser Ala Asp Val 855	860	865	2947
CTG AAA TAC ACG TAT GAC GAT TTC AAA AAG GGA GCC TCA GAT ACT TAT Leu Lys Tyr Thr Tyr Asp Asp Phe Lys Lys Gly Ala Ser Asp Thr Tyr 870	875	880	2995
GTG ACA TAC CTC ATA AGA ACA GAA GAA AAG GGA CGT TCT CAG AGC TTG Val Thr Tyr Leu Ile Arg Thr Glu Glu Lys Gly Arg Ser Gln Ser Leu 885	890	895	3043
TCT GAA GTT TTG AAA TAT GAA ATT GAC GTT GGG AAT GAG TCA ACC ACA Ser Glu Val Leu Lys Tyr Glu Ile Asp Val Gly Asn Glu Ser Thr Thr 900	905	910	3091
CTT GGT TAT TAC AAT GGG AAG CTG GAA CCT CTG GGC TCC TAC CGG GCT Leu Gly Tyr Tyr Asn Gly Lys Leu Glu Pro Leu Gly Ser Tyr Arg Ala 915	920	925	3139
TGT GTG GCT GGC TTC ACC AAC ATT ACC TTC CAC CCT CAA AAC AAG GGG Cys Val Ala Gly Phe Thr Asn Ile Thr Phe His Pro Gln Asn Lys Gly 935	940	945	3187

- 32 -

CTC ATT GAT GGG GCT GAG AGC TAT GTG TCC TTC AGT CGC TAC TCA GAT Leu Ile Asp Gly Ala Glu Ser Tyr Val Ser Phe Ser Arg Tyr Ser Asp 950 955 960	3235
GCT GTT TCC TTG CCC CAG GAT CCA GGT GTC ATC TGT GGA GCG GTT TTT Ala Val Ser Leu Pro Gln Asp Pro Gly Val Ile Cys Gly Ala Val Phe 965 970 975	3283
GGC TGT ATC TTT GGT GCC CTG GTT ATT GTG ACT GTG GGA GGC TTC ATC Gly Cys Ile Phe Gly Ala Leu Val Ile Val Thr Val Gly Gly Phe Ile 980 985 990	3331
TTC TGG AGA AAG AAG AGG AAA GAT GCA AAG AAT AAT CAA GTG TCC TTT Phe Trp Arg Lys Lys Asp Ala Lys Asn Asn Glu Val Ser Phe 995 1000 1005 1010	3379
TCT CAA ATT AAA CCT AAA AAA TCT AAG TTA ATC AGA GTG GAG AAT TTT Ser Gln Ile Lys Pro Lys Lys Ser Lys Leu Ile Arg Val Glu Asn Phe 1015 1020 1025	3427
GAG GCC TAC TTC AAG AAG CAG CAA GCT GAC TCC AAC TGT GGG TTC GCA Glu Ala Tyr Phe Lys Lys Gln Gln Ala Asp Ser Asn Cys Gly Phe Ala 1030 1035 1040	3475
GAG GAA TAC GAA GAT CTG AAG CTT GTT GGA ATT AGT CAA CCT AAA TAT Glu Glu Tyr Glu Asp Leu Lys Leu Val Gly Ile Ser Gln Pro Lys Tyr 1045 1050 1055	3523
GCA GCA GAA CTG GCT GAG AAT AGA GGA AAG AAT CGC TAT AAT AAT GTT Ala Ala Glu Leu Ala Glu Asn Arg Gly Lys Asn Arg Tyr Asn Asn Val 1060 1065 1070	3571
CTG CCC TAT GAT ATT TCC CGT GTC AAA CTT TCG GTC CAG ACC CAT TCA Leu Pro Tyr Asp Ile Ser Arg Val Lys Leu Ser Val Gln Thr His Ser 1075 1080 1085 1090	3619
ACG GAT GAC TAC ATC AAT GCC AAC TAC ATG CCT GGC TAC CAC TCC AAG Thr Asp Asp Tyr Ile Asn Ala Asn Tyr Met Pro Gly Tyr His Ser Lys 1095 1100 1105	3667
AAA GAT TTT ATT GCC ACA CAA GGA CCT TTA CCG AAC ACT TTG AAA GAT Lys Asp Phe Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr Leu Lys Asp 1110 1115 1120	3715
TTT TGG CGT ATG GTT TGG GAG AAA AAT GTA TAT GCC ATC ATT ATG TTG Phe Trp Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile Ile Met Leu 1125 1130 1135	3763
ACT AAA TGT GTT GAA CAG GGA AGA ACC AAA TGT GAG GAG TAT TGG CCC Thr Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu Glu Tyr Trp Pro 1140 1145 1150	3811
TCC AAG CAG GCT CAG GAC TAT GGA GAC ATA ACT GTG GCA ATG ACA TCA Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala Met Thr Ser 1155 1160 1165 1170	3859
GAA ATT GTT CTT CCG GAA TGG ACC ATC AGA GAT TTC ACA GTG AAA AAT Glu Ile Val Leu Pro Glu Trp Thr Ile Arg Asp Phe Thr Val Lys Asn 1175 1180 1185	3907
ATC CAG ACA AGT GAG AGT CAC CCT CTG AGA CAG TTC CAT TTC ACC TCC	3955

- 33 -

Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe His Phe Thr Ser		
1190	1195	1200
TGG CCA GAC CAC GGT GTT CCC GAC ACC ACT GAC CTG CTC ATC AAC TTC		4003
Trp Pro Asp His Gly Val Pro Asp Thr Thr Asp Leu Leu Ile Asn Phe		
1205	1210	1215
CGG TAC CTC GTT CGT GAC TAC ATG AAG CAG AGT CCT CCC GAA TCG CCG		4051
Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln Ser Pro Pro Glu Ser Pro		
1220	1225	1230
ATT CTG CTG CAT TGC AGT GCT GGG GTC GGA AGG ACG GGC ACT TTC ATT		4099
Ile Leu Val His Cys Ser Ala Gly Val Gly Arg Thr Gly Thr Phe Ile		
1235	1240	1245
GCC ATT GAT CGT CTC ATC TAC CAG ATA GAG AAT GAG AAC ACC GTG GAT		4147
Ala Ile Asp Arg Leu Ile Tyr Gln Ile Glu Asn Glu Asn Thr Val Asp		
1255	1260	1265
GTG TAT GGG ATT GTG TAT GAC CTT CGA ATG CAT AGG CCT TTA ATG GTG		4195
Val Tyr Gly Ile Val Tyr Asp Leu Arg Met His Arg Pro Leu Met Val		
1270	1275	1280
CAG ACA GAG GAC CAG TAT GTT TTC CTC AAT CAG TGT GTT TTG GAT ATT		4243
Gln Thr Glu Asp Gln Tyr Val Phe Leu Asn Gln Cys Val Leu Asp Ile		
1285	1290	1295
GTC AGA TCC CAG AAA GAC TCA AAA GTA GAT CTT ATC TAC CAG AAC ACA		4291
Val Arg Ser Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr		
1300	1305	1310
ACT GCA ATG ACA ATC TAT GAA AAC CTT GCG CCC GTG ACC ACA TTT GGA		4339
Thr Ala Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly		
1315	1320	1325
AAG ACC AAT GGT TAC ATC GCC TAATTCCAAA GGAATAACCT TTCT		4384
Lys Thr Asn Gly Tyr Ile Ala		
1335		
GGAGTGAACC AGACCGTCGC ACCCACAGCG AAGGCACATG CCCCCGATGTC GACATGTTTT		4444
TATATGTCTA ATATCTTAAT TCTTTGTTCT GTTTGTGAG AACTAATTTT GAGGGCATGA		4504
AGCTGCATAT GATAGATGAC AAATTGGGGC TGTCGGGGGC TGTGGATGGG TGGGGAGCAA		4564
ATCATCTGCA TTCCTGATGA CCAATGGGAT GAGGTCACTT TTTTTTTTTT CCCCCTTGAG		4624
GATTGCGAA ACCCAGGAAA AGGGATCTAT GATTTTTTTT TCCAAAACAA TTTCTTTTT		4684
AAAAAGACTA TTTTATATGA TTCACATGCT AAAGCCAGGA TTGTGTTGGG TTGAATATAT		4744
TTTAAGTATC AGAGGTCTAT TTTTACCTAC TGTGTCTTGG AATCTAGCCG ATGGAAAATA		4804
CCTAATTGTG GATGATGATT GCGCAGGGAG GGGTACGTGG CACCTCTTCC GAATGGGTTT		4864
TCTATTGAA CATGTGCCTT TTCTGAATTA TGCTTCCACA GGCAAAACTC AGTAGAGATC		4924
TATATTTTG TACTGAATCT CATAATTGGA ATATACGGAA TATTTAAACA GTAGCTTAGC		4984
ATCAGAGGTT TGCTTCCTCA GTAACATTTC TGTTCTCATT TGATCAGGGG AGGCCTCTT		5044

- 34 -

GCCCCGGCCC CGCTTCCCCCT GCCCCGTGT GATTTGTGCT CCATTTTTC TTCCCTTTTC	5104
CCTCCCAGTT TTC	5117

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1337 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Pro Ala Ala Arg Glu Ala Arg Leu Pro Pro Arg Ser Pro Gly	
1 5 10 15	
Leu Arg Trp Ala Leu Pro Leu Leu Leu Leu Leu Arg Leu Gly Gln	
20 25 30	
Ile Leu Cys Ala Gly Gly Thr Pro Ser Pro Ile Pro Asp Pro Ser Val	
35 40 45	
Ala Thr Val Ala Thr Gly Glu Asn Gly Ile Thr Gln Ile Ser Ser Thr	
50 55 60	
Ala Glu Ser Phe His Lys Gln Asn Gly Thr Gly Thr Pro Gln Val Glu	
65 70 75 80	
Thr Asn Thr Ser Glu Asp Gly Glu Ser Ser Gly Ala Asn Asp Ser Leu	
85 90 95	
Arg Thr Pro Glu Gln Gly Ser Asn Gly Thr Asp Gly Ala Ser Gln Lys	
100 105 110	
Thr Pro Ser Ser Thr Gly Pro Ser Pro Val Phe Asp Ile Lys Ala Val	
115 120 125	
Ser Ile Ser Pro Thr Asn Val Ile Leu Thr Trp Lys Ser Asn Asp Thr	
130 135 140	
Ala Ala Ser Glu Tyr Lys Tyr Val Val Lys His Lys Met Glu Asn Glu	
145 150 155 160	
Lys Thr Ile Thr Val Val His Gln Pro Trp Cys Asn Ile Thr Gly Leu	
165 170 175	
Arg Pro Ala Thr Ser Tyr Val Phe Ser Ile Thr Pro Gly Ile Gly Asn	
180 185 190	
Glu Thr Trp Gly Asp Pro Arg Val Ile Lys Val Ile Thr Glu Pro Ile	
195 200 205	
Pro Val Ser Asp Leu Arg Val Ala His Gly Cys Glu Glu Gly Cys Ser	
210 215 220	
Leu Ser Trp Ser Asn Gly Asn Gly Thr Ala Ser Cys Arg Val Leu Leu	
225 230 235 240	

- 35 -

Glu Ser Ile Gly Ser His Glu Glu Leu Thr Gln Asp Ser Arg Leu Gln
245 250 255

Val Asn Ile Ser Asp Leu Lys Pro Gly Val Gln Tyr Asn Ile Asn Pro
260 265 270

Tyr Leu Leu Gln Ser Asn Lys Thr Lys Gly Asp Pro Leu Ala Gln Lys
275 280 285

Val Ala Trp Met Pro Ala Ile Gln Arg Glu Ala Gly Gln Gly Ala Pro
290 295 300

Pro Pro Leu Cys Met Met Ser Pro Phe Val Gly Pro Val Asp Pro Ser
305 310 315 320

Ser Gly Gln Gln Ser Arg Asp Thr Glu Val Leu Leu Val Gly Leu Glu
325 330 335

Pro Gly Thr Arg Tyr Asn Ala Thr Val Tyr Ser Gln Ala Ala Asn Gly
340 345 350

Thr Glu Gly Gln Pro Gln Ala Ile Glu Phe Arg Thr Asn Ala Ile Gln
355 360 365

Val Phe Asp Val Thr Ala Val Asn Ile Ser Ala Thr Ser Leu Thr Leu
370 375 380

Ile Trp Lys Val Ser Asp Asn Glu Ser Ser Ser Asn Tyr Thr Tyr Lys
385 390 395 400

Ile His Val Ala Gly Glu Thr Asp Ser Ser Asn Leu Asn Val Ser Glu
405 410 415

Pro Arg Ala Val Ile Pro Gly Leu Arg Ser Ser Thr Phe Tyr Asn Ile
420 425 430

Thr Val Cys Pro Val Leu Gly Asp Ile Glu Gly Thr Pro Gly Phe Leu
435 440 445

Gln Val His Thr Pro Pro Val Pro Val Ser Asp Phe Arg Val Thr Val
450 455 460

Val Ser Thr Thr Glu Ile Gly Leu Ala Trp Ser Ser His Asp Ala Glu
465 470 475 480

Ser Phe Gln Met His Ile Thr Gln Glu Gly Ala Gly Asn Ser Arg Val
485 490 495

Glu Ile Thr Thr Asn Gln Ser Ile Ile Ile Gly Gly Leu Phe Pro Gly
500 505 510

Thr Lys Tyr Cys Phe Glu Ile Val Pro Lys Gly Pro Asn Gly Thr Glu
515 520 525

Gly Ala Ser Arg Thr Val Cys Asn Arg Thr Val Pro Ser Ala Val Phe
530 535 540

Asp Ile His Val Val Tyr Val Thr Thr Glu Met Trp Leu Asp Trp
545 550 555 560

Lys Ser Pro Asp Gly Ala Ser Glu Tyr Val Tyr His Leu Val Ile Glu

- 36 -

565

570

575

Ser Lys His Gly Ser Asn His Thr Ser Thr Tyr Asp Lys Ala Ile Thr
580 585 590

Leu Gln Gly Leu Ile Pro Gly Thr Leu Tyr Asn Ile Thr Ile Ser Pro
595 600 605

Glu Val Asp His Val Trp Gly Asp Pro Asn Ser Thr Ala Gln Tyr Thr
610 615 620

Arg Pro Ser Asn Val Ser Asn Ile Asp Val Ser Thr Asn Thr Thr Ala
625 630 635 640

Ala Thr Leu Ser Trp Gln Asn Phe Asp Asp Ala Ser Pro Thr Tyr Ser
645 650 655

Tyr Cys Leu Leu Ile Glu Lys Ala Gly Asn Ser Ser Asn Ala Thr Gln
660 665 670

Val Val Thr Asp Ile Gly Ile Thr Asp Ala Thr Val Thr Glu Leu Ile
675 680 685

Pro Gly Ser Ser Tyr Thr Val Glu Leu Phe Ala Gln Val Gly Asp Gly
690 695 700

Ile Lys Ser Leu Glu Pro Gly Arg Lys Ser Phe Cys Thr Asp Pro Ala
705 710 715 720

Ser Met Ala Ser Phe Asp Cys Glu Val Val Pro Lys Glu Pro Ala Leu
725 730 735

Val Leu Lys Trp Thr Cys Pro Pro Gly Ala Asn Ala Gly Phe Glu Leu
740 745 750

Glu Val Ser Ser Gly Ala Trp Asn Asn Ala Thr His Leu Glu Ser Cys
755 760 765

Ser Ser Glu Asn Gly Thr Glu Tyr Arg Thr Glu Val Thr Tyr Leu Asn
770 775 780

Phe Ser Thr Ser Tyr Asn Ile Ser Ile Thr Thr Val Ser Cys Gly Lys
785 790 795 800

Met Ala Ala Pro Thr Arg Asn Thr Cys Thr Thr Gly Ile Thr Asp Pro
805 810 815

Pro Pro Pro Asp Gly Ser Pro Asn Ile Thr Ser Val Ser His Asn Ser
820 825 830

Val Lys Val Lys Phe Ser Gly Phe Glu Ala Ser His Gly Pro Ile Lys
835 840 845

Ala Tyr Ala Val Ile Leu Thr Thr Gly Glu Ala Gly His Pro Ser Ala
850 855 860

Asp Val Leu Lys Tyr Thr Tyr Asp Asp Phe Lys Lys Gly Ala Ser Asp
865 870 875 880

Thr Tyr Val Thr Tyr Leu Ile Arg Thr Glu Glu Lys Gly Arg Ser Gln
885 890 895

- 37 -

Ser Leu Ser Glu Val Leu Lys Tyr Glu Ile Asp Val Gly Asn Glu Ser
900 905 910

Thr Thr Leu Gly Tyr Tyr Asn Gly Lys Leu Glu Pro Leu Gly Ser Tyr
915 920 925

Arg Ala Cys Val Ala Gly Phe Thr Asn Ile Thr Phe His Pro Gln Asn
930 935 940

Lys Gly Leu Ile Asp Gly Ala Glu Ser Tyr Val Ser Phe Ser Arg Tyr
945 950 955 960

Ser Asp Ala Val Ser Leu Pro Gln Asp Pro Gly Val Ile Cys Gly Ala
965 970 975

Val Phe Gly Cys Ile Phe Gly Ala Leu Val Ile Val Thr Val Gly Gly
980 985 990

Phe Ile Phe Trp Arg Lys Lys Arg Lys Asp Ala Lys Asn Asn Glu Val
995 1000 1005

Ser Phe Ser Gln Ile Lys Pro Lys Lys Ser Lys Leu Ile Arg Val Glu
1010 1015 1020

Asn Phe Glu Ala Tyr Phe Lys Lys Gln Gln Ala Asp Ser Asn Cys Gly
1025 1030 1035 1040

Phe Ala Glu Glu Tyr Glu Asp Leu Lys Leu Val Gly Ile Ser Gln Pro
1045 1050 1055

Lys Tyr Ala Ala Glu Leu Ala Glu Asn Arg Gly Lys Asn Arg Tyr Asn
1060 1065 1070

Asn Val Leu Pro Tyr Asp Ile Ser Arg Val Lys Leu Ser Val Gln Thr
1075 1080 1085

His Ser Thr Asp Asp Tyr Ile Asn Ala Asn Tyr Met Pro Gly Tyr His
1090 1095 1100

Ser Lys Lys Asp Phe Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr Leu
1105 1110 1115 1120

Lys Asp Phe Trp Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile Ile
1125 1130 1135

Met Leu Thr Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu Glu Tyr
1140 1145 1150

Trp Pro Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala Met
1155 1160 1165

Thr Ser Glu Ile Val Leu Pro Glu Trp Thr Ile Arg Asp Phe Thr Val
1170 1175 1180

Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe His Phe
1185 1190 1195 1200

Thr Ser Trp Pro Asp His Gly Val Pro Asp Thr Thr Asp Leu Leu Ile
1205 1210 1215

Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln Ser Pro Pro Glu

- 38 -

1220	1225	1230
Ser Pro Ile Leu Val His Cys Ser Ala Gly Val Gly Arg Thr Gly Thr		
1235	1240	1245
Phe Ile Ala Ile Asp Arg Leu Ile Tyr Gln Ile Glu Asn Glu Asn Thr		
1250	1255	1260
Val Asp Val Tyr Gly Ile Val Tyr Asp Leu Arg Met His Arg Pro Leu		
1265	1270	1275
Met Val Gln Thr Glu Asp Gln Tyr Val Phe Leu Asn Gln Cys Val Leu		
1285	1290	1295
Asp Ile Val Arg Ser Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr Gln		
1300	1305	1310
Asn Thr Thr Ala Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr Thr		
1315	1320	1325
Phe Gly Lys Thr Asn Gly Tyr Ile Ala		
1330	1335	

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Lys Cys Ala Gln Tyr Trp Pro
1 5

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

His Cys Ser Ala Gly Ile Gly
1 5

- 39 -

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AARTGYGCNC ARTAYTGGCC

20

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE

(D) OTHER INFORMATION: /note= "Base designated N at position 6 is Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCDATNCCNG CRCTRCARTG

20

- 40 -

WHAT IS CLAIMED IS:

1. A purified and isolated density enhanced protein tyrosine phosphatase polypeptide.
2. A receptor-like protein tyrosine phosphatase polypeptide according to claim 1.
3. A Type III receptor-like protein tyrosine phosphatase polypeptide according to claim 1.
4. The Type III receptor-like protein tyrosine phosphatase polypeptide according to claim 3 consisting essentially of the huDEP-1 amino acid sequence set out in SEQ ID NO: 2, or a variant thereof.
5. A polynucleotide encoding the protein tyrosine phosphatase polypeptide of claim 1.
6. The polynucleotide of claim 5 which is a DNA.
7. The DNA of claim 6 which is selected from the group consisting of genomic DNA, cDNA, partially chemically synthesized DNA, and wholly chemically synthesized DNA.
8. The DNA of claim 6 further comprising regulatory DNA sequences which direct transcription of the DNA.
9. A DNA expression construct comprising the DNA of claim 8.

- 41 -

10. A host cell transformed or transfected with the DNA of claim 6.

11. A method for producing a density enhanced protein tyrosine phosphatase polypeptide comprising growing a host cell according to claim 10 in a suitable medium and isolating the phosphatase polypeptide from the host cell or the medium of its growth.

12. A purified and isolated polynucleotide encoding a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide.

13. The polynucleotide according to claim 12 which is a DNA.

14. The DNA of claim 13 which is selected from the group consisting of cDNA, genomic DNA, partially chemically synthesized DNA, and wholly chemically synthesized DNA.

15. The DNA of claim 13 comprising a huDEP-1 protein coding sequence as set forth in SEQ ID NO: 1, or a variant thereof.

16. The DNA of claim 13 further comprising regulatory DNA sequences which direct transcription of the DNA.

17. A purified and isolated polynucleotide selected from the group consisting of:

- a) the DNA sequence set out in SEQ ID NO: 1, and
- b) a DNA molecule which hybridizes under stringent conditions to the protein coding portion of the DNA of (a).

- 42 -

18. A DNA encoding a huDEP-1 amino acid sequence set out in SEQ ID NO: 2, or a variant thereof.

19. A DNA expression construct comprising the DNA of claim 16.

20. A host cell transformed or transfected with the DNA of claim 13.

21. A method for producing a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide comprising the steps of growing the host cell according to claim 20 in a suitable medium and isolating the polypeptide from the host cell or the medium of its growth.

22. The method of claim 21 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase is huDEP-1, or a variant thereof.

23. A polypeptide or peptide capable of specifically binding to a density enhanced protein tyrosine phosphatase polypeptide.

24. A polypeptide or peptide capable of specifically binding to a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide.

25. The polypeptide according to claim 24 which is an antibody.

- 43 -

26. The antibody according to claim 25 which is a monoclonal antibody.

27. An anti-idiotype antibody specific for the monoclonal antibody of claim 26.

28. A hybridoma cell line producing the antibody of claim 26 or 27.

29. The polypeptide or peptide according to claims 24, 25, 26, or 27 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide is huDEP-1, or a variant thereof.

30. A method for isolating a polynucleotide encoding a polypeptide that binds to a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide comprising the steps of:

a) transforming or transfecting host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA-binding domain and an activating domain;

b) expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide and either the DNA-binding domain or the activating domain of the transcription factor;

c) expressing in the host cells a library of second hybrid DNA sequences encoding second fusions of part or all of putative density enhanced Type III receptor-like protein tyrosine phosphatase binding polypeptides and either the activating domain or DNA binding domain of the transcription factor which is not incorporated in the first fusion;

- 44 -

- d) transforming or transfecting the host cells with a DNA construct comprising a protein tyrosine kinase gene;
- e) detecting binding of density enhanced Type III receptor-like protein tyrosine phosphatase binding polypeptide(s) to the phosphatase polypeptide in the host cell by detecting the production of reporter gene product in the host cell(s); and
- f) isolating the second hybrid DNA sequences encoding the phosphatase binding polypeptide from the host cell(s).

31. The method of claim 30 wherein the promoter is the beta-galactosidase promoter, the DNA-binding domain is the *lexA* DNA-binding domain, the activating domain is the *GAL4* transactivation domain, the reporter gene is the *lacZ* gene and the host cells are yeast host cells.

32. The method according to claim 30 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase is huDEP-1, or a variant thereof.

33. The method according to claim 30 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase is a catalytically inactive variant of huDEP-1 capable of binding huDEP-1 substrate.

34. A method for detecting proteins which bind to a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide isoform comprising the steps of:

- a) transforming or transfecting host cells with a hybrid DNA sequence encoding a fusion between a putative phosphatase binding protein and a ligand capable of high affinity binding to a specific counterreceptor;

- 45 -

- b) expressing the hybrid DNA sequence in the host cells under appropriate conditions;
- c) immobilizing fusion protein from the host cells by exposing the fusion protein to the specific counterreceptor in immobilized form;
- d) contacting the density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide with the immobilized fusion protein; and
- e) detecting the phosphatase polypeptide bound to the fusion protein using a reagent specific for the phosphatase polypeptide.

35. The method of claim 34 wherein the ligand is glutathione-S-transferase and the counterreceptor is glutathione.

36. The method of claim 34 wherein the ligand is hemagglutinin and the counterreceptor is a hemagglutinin-specific antibody.

37. The method of claim 34 wherein the ligand is polyhistidine and the counterreceptor is nickel.

38. The method of claim 34 wherein the ligand is maltose-binding protein and the counterreceptor is amylose.

39. The method of claim 33 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide is huDEP-1, or a variant thereof.

1 / 3

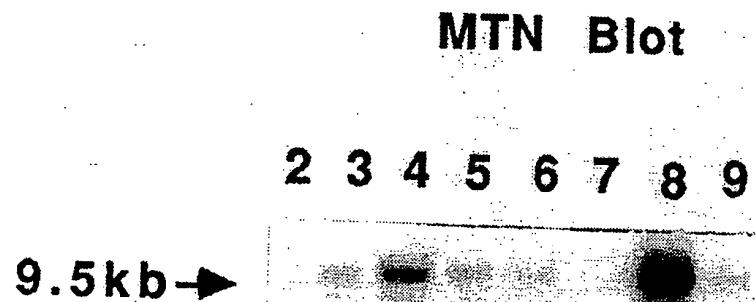


FIG. 1A

2 / 3

MTN Blot II

2 3 4 5 6 7 8 9

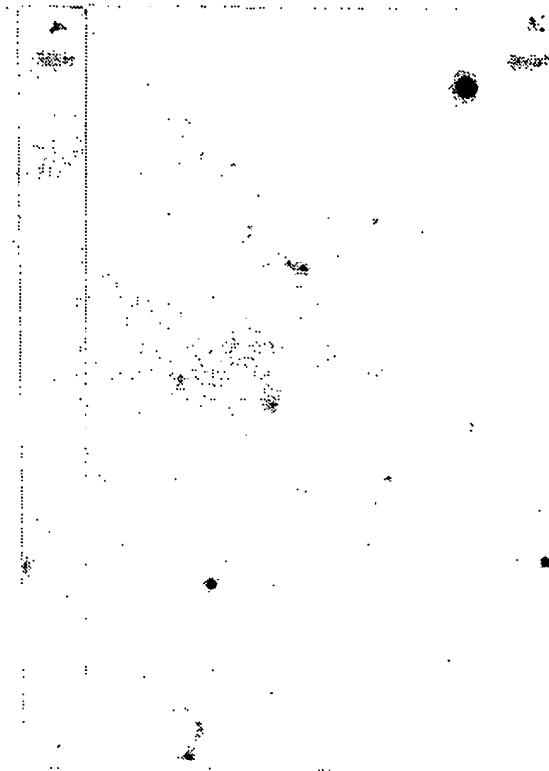


FIG. 1B

SUBSTITUTE SHEET (RULE 26)

3 / 3

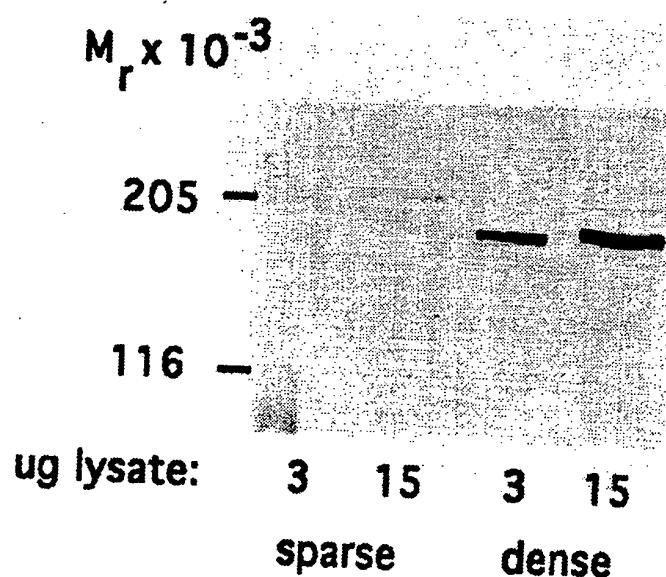


FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/05512

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/55 C12N9/16 C12N5/10 C07K16/40 C07K16/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 88, August 1991 WASHINGTON US, pages 6996-7000, PALLEN AND TONG 'Elevation of membrane tyrosine phosphatase activity in density-dependent growth-arrested fibroblasts' cited in the application see the whole document</p> <p>---</p> <p>-/-</p>	1,23

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "V" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

1 Date of the actual completion of the international search

26 July 1995

Date of mailing of the international search report

16.08.95

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Authorized officer

Cupido, M

INTERNATIONAL SEARCH REPORT

Inten	nal Application No
PCT/US 95/05512	

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY , vol. 269, no. 3, 21 January 1994 MD US, pages 2075-2081, MATOZAKI ET AL. 'Molecular cloning of a human transmembrane-type protein tyrosine phosphatase and its expression in gastrointestinal cancers' cited in the application see the whole document ---	1-39
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, October 1994 WASHINGTON US, pages 9680-9684, ÖSTMAN ET AL. 'Expression of DEP-1, a receptor-like protein-tyrosine-phosphatase, is enhanced with increasing cell density' see the whole document ---	1-39
X,P	BLOOD, vol. 84, no. 12 , 15 December 1994 pages 4186-4194, HONDA ET AL. 'Molecular cloning, characterization, and chromosomal location of a novel protein-tyrosine phosphatase, HPTPeta' see figure 3 which has over 99% identity with SEQ ID NO:1 -----	1-39